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Seed-priming of sorghum with antifungal extracts from *Balanites aegyptiaca* and *Eclipta alba* in different agro-ecological zones of Burkina Faso

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Seed-priming of sorghum with an aqueous extract from the herbal plant, *Eclipta alba* has previously been found to increase crop yield of sorghum in Burkina Faso dependent on field location. In the present study, a 2.5% w/v antifungal extract from the desert tree, *Balanites aegyptiaca*, was similarly shown to increase the yield of sorghum by seed treatment. The effect was compared to the effect of *E. alba* extract on different types of seeds in different locations. A participatory trial including forty-six fields in three agro-ecological zones was conducted using local, farm-saved seeds. The overall effect on yield conferred by the *B. aegyptiaca* extract was significantly higher than the effect conferred by the *E. alba* extract (+31% versus +21%, *p*<0.03). However, in one zone the opposite hierarchy was observed; also when formally propagated, seeds were used for testing. The same, South-Eastern zone was characterized by poor crop performance despite a relatively high rainfall. Antifungal activity was confirmed in both extracts *in vitro* and different levels of protection against the pathogen *Curvularia lunata* were demonstrated in seedlings. The findings are encouraging for a regionally differential use of botanicals in seed treatment and more research to understand local differences in the crop response is suggested.

Key words: Bio-priming, *sorghum bicolor*, emergence, mycoflora, *Epicoccum*, *Fusarium*.

INTRODUCTION

In Burkina Faso, seeds of sorghum are commonly infested by pathogenic ascomycetes such as *Epicoccum sorghinum* (prev. *Phoma sorghina*), *Fusarium thapsinum* (prev. *F. moniliforme*) and several species of *Curvularia* (*Curvularia lunata*, *Curvularia alcornii* and others) (Prom et al., 2003; Zida et al., 2008a; Stokholm et al., 2016). Soaking of sorghum seeds for 6 h in 2.5% w/v aqueous extract of *Eclipta alba* reduced symptoms of fungal infection in seedlings and yield was increased by approximately 20% in a field trial involving multiple locations (Zida et al., 2015). Subsequently, it was recently found that the yield enhancing effect of *E. alba*

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extract is likely to be caused by a dual mechanism of seed hydropriming and suppression of fungal pathogens (Zida et al., 2018). The plant *E. alba* (L.) Hassk. (syn.: *Eclipta prostrata* Eng.: False daisy) is known as an annual weed in the family Asteraceae and is found globally in humid tropical and subtropical areas (Holm et al., 1977). Line drawings for its identification, photographs and distribution maps can be found online in CABI compendium of invasive species (CABI, 2018). *E. alba* has a strong record in ethno-botanical medicine and cosmetology (Bhalerao et al., 2013; Jahan et al., 2014; Begum et al., 2015). Several complex alkaloids have been identified in *E. alba* and antifungal activity is attributed to the compound 25-*β*-hydroxyverazine (Abdel-Kader et al., 1998).

In Burkina Faso, *E. alba* is commonly found in Central and Southern parts of the country, where humidity occurs at least periodically (Zida et al., 2008b). A country-wide field trial showed a moderate ability of *E. alba* extract to increase yield of sorghum (Zida et al., 2012). However, the effect of *E. alba* extract is highly dependent on the field location with a significant tendency of a stronger effect found on fields showing a low emergence of sorghum (Zida et al., 2016). Thus, a strategy of using *E. alba* extract for seed treatment in Burkina Faso faces two major challenges: (1) the plant is not commonly found in the northern parts of the country and (2) the effect is highly variable between locations. In order to mitigate these challenges, the objective of the present study was to test an alternative extract from the plant, *Balanites aegyptiaca*, found widely in Burkina Faso and for which antifungal activity is also known (Bonzi et al., 2012).

B. aegyptiaca Del. (family Zygophyllaceae; Eng.: Desert date) is a thorny tree distributed mainly in drylands of sub-Saharan Western Africa (Arbonnier, 2002). A distribution map and pictures for its identification can be found online (GBIF, 2018). Like *E. alba*, the *B. aegyptiaca* tree is a well-known medicinal plant, with a high number of complex compounds including saponins identified (Chothani and Vaghasiya, 2011). To our knowledge, none of them have yet been attributed directly to antifungal activity. In the present study, testing of the *B. aegyptiaca* aqueous bark extract in seed treatment included direct comparison to water (hydropriming) and to *E. alba* extract by testing on multiple locations across different agro-ecological zones. Both farm-saved and formally propagated seeds were used as testing material. The study area of field experiments included three different sorghum-growing regions: (A) Northern, (B) Central, and (C) South-Eastern Region (Figure 1). The Northern region (Zone A) is characterized by low rainfall (less than 600 mm annually) and dry-land agriculture including sorghum as the second most important crop next to pearl millet (INSD, 2016; De Longueville et al., 2016). The Central region (Zone B) consists of the capital, Ouagadougou, and has an annual rainfall between 600 and 900 mm. In

Figure 1. The study area. Fourteen locations (villages) of field test sites distributed across three major agro-ecological zones of sorghum are shown. A) Northern Zone. B) Central Zone. C) South-Eastern Zone. Fifty-years isohyets of 600 mm and 900 mm are indicated by colors according to De Longueville et al. (2016). In the participatory field trial in 2017 involving 46 farmers all the fourteen locations shown were included with 2-4 farmers and fields included in each village.

the rural districts of zone B sorghum is the main crop (INSD, 2016). Zone C (the South-Eastern area) has an annual rainfall close to 900 mm. This zone contains the highest biodiversity of wild plant species in Burkina Faso (Schmidt et al., 2005) and sorghum is the main crop followed by maize (INSD, 2016).

Fourteen locations (villages) of field test sites distributed across three major agro-ecological zones of sorghum are shown: (A) Northern Zone; (B) Central Zone; (C) South-Eastern Zone. Fifty-years isohyets of 600 and 900 mm are indicated by colors according to De Longueville et al. (2016). In the participatory field trial in 2017 involving 46 farmers, all the fourteen locations shown were included with 2 to 4 farmers and fields included in each village.

In addition to field trials, several experiments were carried out *in vitro* to compare the two plant extracts with regard to antifungal effect and protection of sorghum seedlings from fungal seed inoculum.

MATERIALS AND METHODS

Seeds

In the field trial with 36 field tests comparing treatment with *B. aegyptiaca* extract and hydropriming, seeds of two varieties of sorghum were included: Kouria (13 field tests) and Kapelga (23 field tests). Both varieties were propagated by the Institute of Environment and Agricultural Research (IN.E.R.A, Burkina Faso). In the subset of 21 experiments including also treatment with *E. alba* extract only seeds of variety Kapelga were included. In the field trial with farm-saved seeds, forty-six seed samples from each of forty-six farmers from a total of 14 different villages were used (14

Table 1. *B. aegyptiaca* extract tested against hydropriming in seed treatment of sorghum (formal seeds).

Year	N	Yield parameter	Treatment			ANOVA
			NoT	H_2O	Ba2.5%	One-way
2015	9	Mean kg/ha	942	1231	1464	
2017	27	Mean kg/ha	837	941	937	
Total	36	Mean kg/ha	863	1014	1069	
		Field average (%)*	87.8	101.1	109.9	<0.00006
		Yield increase %	-	+15.1%	+25.2%	
		p(NoT)	-	<0.009	<0.00004	
		$p(H_2O)$	-	-	<0.04	

N = Number of field test, NoT = No Treatment, H_2O = Hydropriming, Ba= *B. aegyptiaca* extract. Field experiments were carried out on varieties Kouria and Kapelga in Northern, Central and South-Eastern parts of Burkina Faso.
*Yields in NoT-plots varied from 130-2800 kg/ha between fields. To avoid a bias from high-yielding fields, statistics were made only on field averaged values (%).

villages is as shown in Figure 1). Two to four farmers from each village were included. Each seed sample was tested only on the farmers own field. For experiments *in vitro*, seeds of the cultivar Kapelga (sample 49.071) propagated by IN.E.R.A and farm-saved seeds from the village Diapangou (sample 40.066) were used as indicated.

Plant extracts

Wild plants of *E. alba* were collected by uprooting (pulling by hand) of plants growing in humid sites in Central Burkina Faso. Soil particles on roots were removed by rinsing in tap water. Barks of *B. aegyptiaca* were collected from wild trees growing in fields near farmers' homes in several parts of Burkina Faso. Barks (soft parts) were removed from the trunk of the tree with the help of a pick. The material of both plants collected was air-dried, ground with a mortar into powder, and sieved with a mesh of approximately 2 mm diameter. Plant powders were stored in sealed plastic bags in darkness at room temperature until use. The preparation of aqueous extracts of *E. alba* and *B. aegyptiaca* followed the same procedure: the powder of both plants was mixed with distilled water at the concentration 2.5% (W/V) and incubated for passive extraction at 25 to 30°C for 20 h. Aqueous extracts were obtained by filtering the mixtures with pieces of cloth (hand filtering) and used the same day (no storing).

Seed treatments

Seeds used for field experiments were either not treated (NoT), soaked for 6 h in distilled water (H_2O) or in either of the two extracts. *E. alba* and *B. aegyptiaca* extracts were applied by soaking of seeds for 6 h in 2.5% extract of each plant. After treatment, seeds were dried at room temperature for one day before sowing. For seeds used in experiments *in vitro*, some were heat treated in a water bath at 55°C for 40 min as indicated (Hot Water, HW) in order to reduce the natural inoculum of seed borne fungi.

Field experiments

Experimental plots were prepared and managed as previously described (Zida et al., 2016). Shortly, for each treatment an experimental plot of at least 25 rows (80 cm spacing, 5 m length) was prepared per field in a random block design. Mineral fertilizer (NPK 14-23-14, 100 kg/ha) and urea (50 kg/ha) were applied. Sowing was done in May-June and harvesting was done in November-December according to local weather forecasts.

In the trial of 36 experiments testing *B. aegyptiaca* extract and hydropriming on formally propagated seeds (Table 1) experimental fields were distributed across all three zones (A, B and C; Figure 1). In the subset of 21 experiments including *E. alba* extract (Table 2) only fields in Zones B (11 fields) and C (10 fields) were included. In the participatory field trial including 46 farmers (Table 3) fields were located in 14 different villages distributed across three zones A, B and C as detailed in Figure 1. For each experimental plot, emergence was determined visually by counting the percentage of seed holes populated with emerging plants, 3 to 4 weeks after sowing. At harvest, grains were collected from each plot and yield was determined after 2 weeks of sun drying of the harvested grain. Data of annual precipitation were recorded at meteorological stations at Titao (Zone A), Ouagadougou (Zone B) and Diapangou (Zone C).

Fungal analysis

Mycoflora of seeds was enumerated by blotter test (Mathur and Kongsdal, 2003). Effect of seed treatments was assessed on the seed sample (variety Kapelga) used for field trials in 2017. Following seed treatments as described earlier (NoT, H_2O, *E. alba* 2.5% and *B. aegyptica* 2.5%) seeds were incubated for 7 days before enumeration of mycoflora. For each treatment, two replicas of the blotter test (each of 100 seeds) were conducted. For farm saved seed sample 49.066, 200 seeds were analyzed by blotter test and found to carry a natural inoculum (% seeds infected) of phytopathogenic fungi: *E. sorghinum* (53%); *Curvularia* species (28%), *Fusarium* species (13%).

Inoculation of seeds *in vitro*

Seeds of variety Kapelga (sample 49.071) were heat treated as described earlier (55°C hot water for 40 min) to eliminate natural fungal inoculum. After this treatment, seeds were divided in two groups: one group was inoculated with a spore suspension of *C. lunata* var. *aeria* (10^5 spores per ml, 15 min) and the second group was soaked in pure water as a control. Seeds were then air-dried and stored for at least 3 days before further treatment (H_2O, Ea2.5%, Ba2.5%) as described earlier or heat treated again (55°C, 40 min) as an anti-fungal control. The isolate of *C. lunata* was isolated from seeds of sorghum from Burkina Faso and identified by

Table 2. Hydropriming, *E. alba* and *B. aegyptiaca* extracts compared in two zones (Sorghum Yield, 2017).

Zone	N	Yield parameter	Treatment				ANOVA
			NoT	H_2O	Ea2.5%	Ba2.5%	One-way
B: Central	11	Mean kg/ha	844	966	889	930	Ns
		Field average (%)	94.9	105.9	97.8	101.4	
		Increase %	-	+11.6	+3.0	+6.9	
C: South-East.	10	Mean kg/ha	687	819	1013	730	<0.012
		Field average (%)	85.9	95.7	111.2	103.1	
		Increase %*	-	+11.3	+29.4	+20.0	
		p(NoT)*	-	Ns	<0.0092	<0.046	
		p(H_2O)*	-	-	<0.042	Ns	

N = Number of field tests, NoT = No Treatment, H_2O = Hydropriming, Ea= *E. alba*, Ba = *B. aegyptiaca*, ns = not significant. A total of 21 field test were performed on formally propagated seeds of sorghum variety Kapelga.
*Increase of yield (%) and statistics are based on field averaged values.

Table 3. Comparison of *E. alba* and *B. aegyptiaca* extracts on farm-saved seeds in three zones (Yield).

Zone	N	Yield parameter	Treatment			ANOVA
			NoT	Ea2.5	Ba2.5	One-way
A Northern	18	Mean kg/ha	529	629	742	<0.0004
		Field Av. %	83.2	98.6	118.1	
		% Incr.	-	+18.5%	+41.9%	
		p(NoT)	-	<0.03	<0.00009	
		p(Ea2.5)	-	-	<0.03	
B Central	15	Mean kg/ha	622	711	756	Ns
		Field Av. %	87.2	103.0	109.8	
		% Incr.	-	+18.1%	+25.9%	
C South-East.	13	Mean kg/ha	560	833	700	<0.009
		Field Av. %	85.6	109.3	105.1	
		% Incr.	-	+27.7%	+22.8%	
		p(NoT)	-	<0.009	<0.03	
		p(Ea2.5)	-	-	Ns	
Total	46	Mean kg/ha	568	713	735	<0.00002
		Field Av. %*	85.2[a]	103.1[b]	111.7[c]	
		% Incr.	-	+21.0%	+31.1%	
		p(NoT)	-	<0.0003	<0.00005	
		p(Ea2.5)	-	-	<0.03	

N = Number of farmers, NoT = No Treatment, Ea = *Eclipta alba*, Ba = *Balanites aegyptiaca,* ns = not significant. *Figures in same row with same letters are not significantly different (Mann-Whitney paired test, p<0.05).

sequencing of *ITS2* region found identical to sequence KF218632 of isolate Curv-Bi-02 (Stokholm et al., 2016).

Analysis of seedlings *in vitro*

Seeds of sorghum (artificially inoculated, sample 49.071 or farm-saved seeds, sample 49.066 carrying a natural inoculum) were treated as described earlier (NoT; H_2O; *E. alba* 2.5%; *B. aegyptica* 2.5%; hot water treatment). After drying of seeds overnight, seedlings were grown from seeds in Hoagland medium as follows: the seeds were sown in 80 ml test-tubes (1 seed per tube) containing 20 ml solidified sterile Hoagland's plant growth medium no. 2. The plants were incubated in a climate chamber at 25°C/16°C day/night temperature and a light/dark ratio of 14/10 h using a continuous photon flux density of 58 to 65 µmol s^{-1} m^{-1} photosynthetically active radiation (PAR) for 16 days after sowing. At 16 days after sowing, dry weight of individual l shoots was

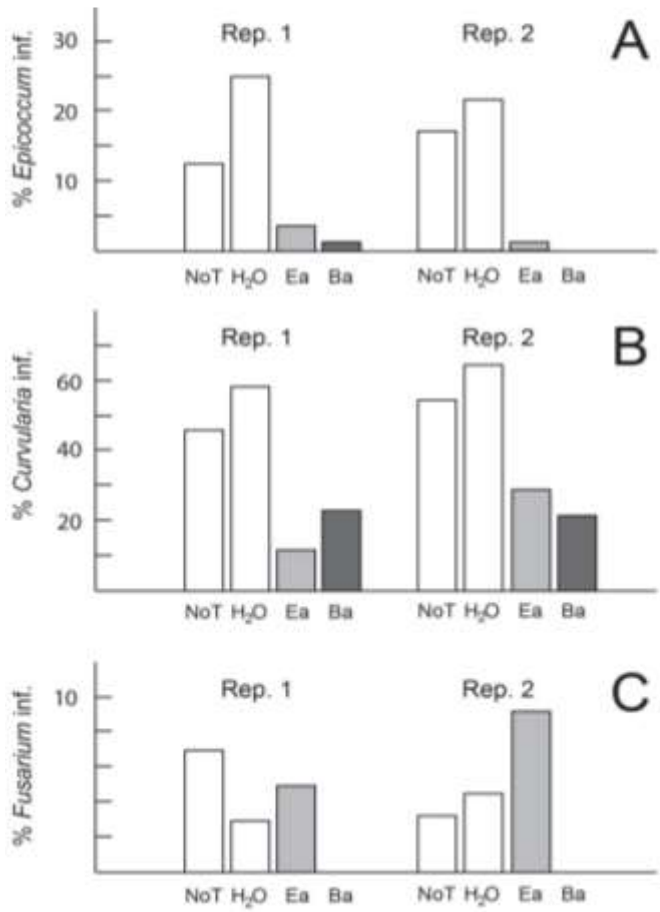

Figure 2. Effect of plant extracts on seed mycoflora. Two repetitions of blotter test showing % of seeds infected by genera (A) *Epicoccum*, (B) *Curvularia* and (C) *Fusarium*. Each replicate consist of 100 formally produced seeds (Kapelga).

measured. For farm-saved seeds (sample 49.066) carrying a natural fungal inoculum, root health was visually assessed by recording of presence/absence of black necrotic tissue. In the experiment with inoculation of *C. lunata* (Figure 3), two repetitions (2 × 24 seeds) were sown per treatment. In the experiment with farm-saved seeds (Figure 4), three repetitions (3 × 24 seeds) were sown per treatment. Before statistical analysis of experiments, 4 non-germinated or poorly germinated seeds/seedlings were removed for each treatment in each repetition.

Statistics

For each plot the yield was calculated in two ways: Absolute yield = kg/ha and relative yield (%) compared to the field average (the average yield of all plots of all treatments on the same field). Simple means of both types of yield were calculated. In order to eliminate field-to-field variation of the general yield level, one-way analysis of variance (ANOVA) was calculated for field averaged yields (%) using software PAST version 3.20 (Hammer et al., 2001). For all data sets with an overall significant ANOVA *p*-value (<0.05) pairwise comparison of each group was done by Mann-Whitney analysis (yield) or Wilcoxon analysis (emergence) using the aforementioned software. For comparison of individual treatments *in vitro*, data of shoot weight were analyzed by Mann-Whitney paired test and data for presence or absence of root necrosis were analyzed by Chi-square test (PAST version 3.20).

RESULTS

B. aegyptiaca extract compared to hydropriming

In two growing seasons, seed treatment with 2.5% *B. aegyptiaca* extract was compared to soaking of seeds in pure water (H_2O, hydropriming) and no treatment (NoT). Formally produced seeds were used as testing material in a total of thirty-six field experiments distributed across the Northern, Central and South-Eastern parts of the country. The total mean of yield (both absolute kg/ha and calculated as field average %) is shown in Table 1. For the field averaged values, one-way ANOVA was significant across all 36 experiments and the following hierarchy of yields was obtained: Ba2.5% > H_2O > NoT. Pairwise comparison of Ba2.5% relative to H_2O (field averaged values) showed a significantly stronger effect of the plant extract: p (H_2O)<0.04. In conclusion, seed treatment with the *B. aegyptica* extract was found to confer a specific increase of yield in Burkina Faso compared to controls of pure water and no treatment.
Field experiments were carried out on varieties Kouria and Kapelga in Northern, Central and South-Eastern parts of Burkina Faso.*Yields in NoT-plots varied from 130 to 2800 kg/ha between fields. To avoid a bias from high-yielding fields, statistics were made only on field averaged values (%).

Effect of *B. aegyptiaca* and *E. alba* extracts compared between two zones (formal seeds)

For the year 2017, a subset of the 27 field experiments described in Table 1 also included plots with seeds treated with *E. alba* extract. This subset of 21 experiments was re-analyzed by inclusion of data for *E. alba* and was stratified into zones B and C in order to reveal any zonal effect (Table 2).

A total of 21 field tests were performed on formally propagated seeds of sorghum variety Kapelga. Increase of yield (%) and statistics are based on field averaged values.

A significant difference between treatments was found by ANOVA only in the low-yielding, Zone C, with the observed hierarchy of treatments: Ea2.5% > Ba2.5% > H_2O> NoT. Both extracts were significantly more efficient than no treatment in Zone C, but only *E. alba* extract performed significantly better than hydropriming (p<0.042). Notably, the same effect of hydropriming was observed in the two zones (ca 11%) and in Zone B; this was the strongest effect observed for any of the treatments.

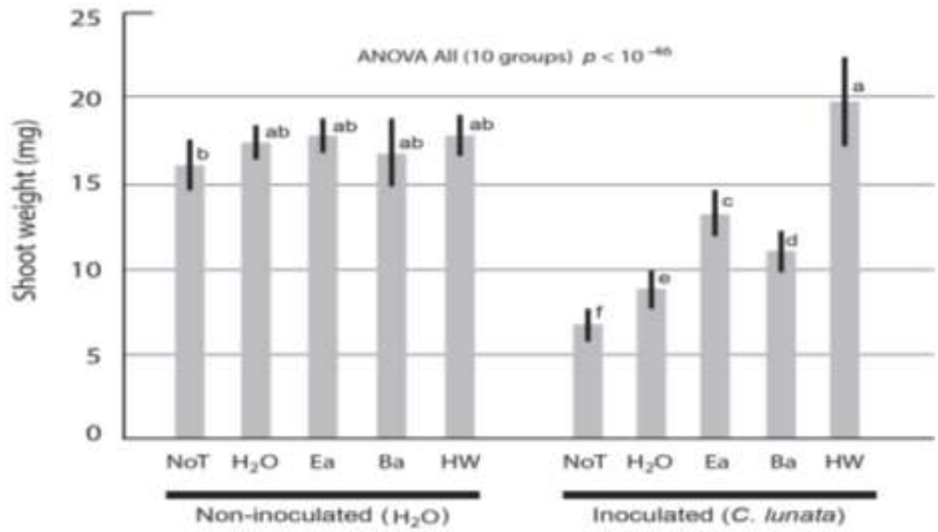

Figure 3. Seedlings growth *in vitro* of artificially inoculated seeds (sample 49.071). Five different treatments: NoT, H_2O, Ea2.5%, Ba2.5% and Hot Water (HW) were compared on seeds inoculated with *C. lunata* or with water (non-inoculated control). For each treatment a total of 2×24 = 48 seeds were tested. Mean shoot dry weight (large bars) and 95% confidence intervals (small bars) are shown. Columns with same letters are not significantly different (Mann Whitney paired test, p< 0.05).

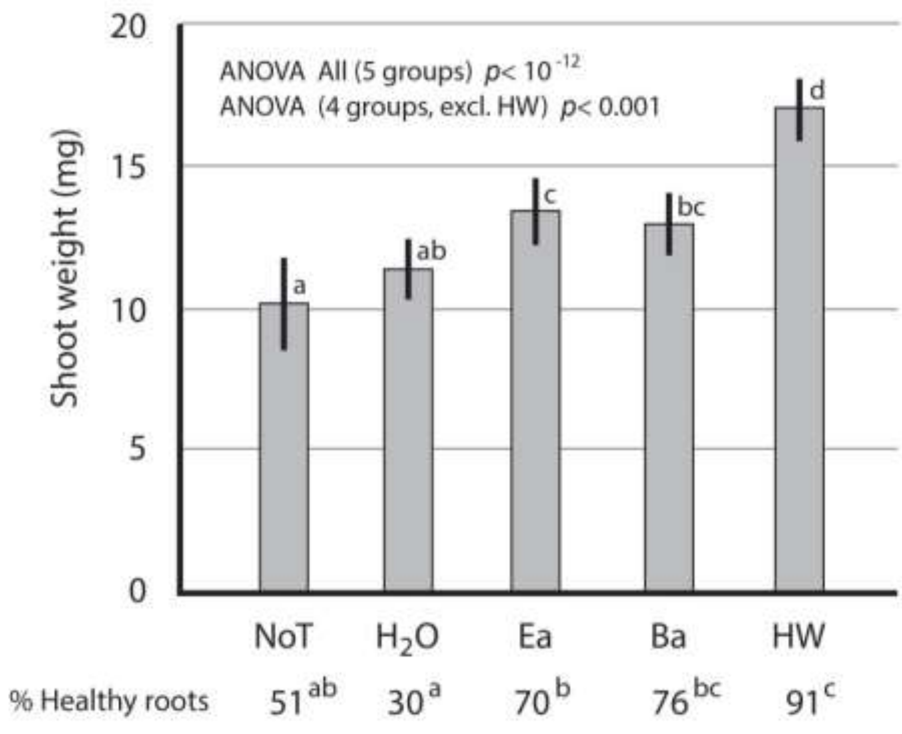

Figure 4. Seedling growth *in vitro* of naturally infected seeds (farm-saved 49.066). Five treatments: NoT, H_2O, Ea2.5%, Ba2.5% and Hot Water (HW) were compared. For each treatment a total of 3×24 = 72 seeds were tested. Mean shoot dry weight (large bars) and 95% confidence intervals (small bars). Columns with same letters are not significantly different (Mann Whitney paired test, p< 0.05). Percentage of healthy roots (absence of dark necrosis) is shown. Groups with same letters are not significantly different (Chi-square test, p<0.05).

Table 4. Rainfall and emergence of farm-saved seeds tested with two plant extracts in three zones.

Zone (Rainfall)	N	Emergence % (means)			ANOVA
		NoT	Ea2.5%	Ba2.5%	
A) Northern (633 mm)	18	63.1	68.5	68.3	ns
B) Central (710 mm)	15	74.8	77.9	79.4	ns
C) South-Eastern (949 mm)	13	63.1	69.5	69.3	ns
Total (direct mean)	46	67.2	72.2	72.1	ns
Total (Field averaged %)	-	95.8	101.7	102.5	$p<0.004$
p-value (paired, NoT)*	-	-	<0.0003	<0.006	-

*Wilcoxon paired test.

Plant extracts tested on farm-saved seeds in three zones

A large scale, participatory field trial was conducted to test the effect of plant extracts on farmers own seeds in their own fields. The trial included 46 farmers distributed in three agricultural zones as shown in Figure 1. The effect of the two extracts was compared to No Treatment. Data were recorded for yield (Table 3), emergence and rainfall (Table 4). An overall significant difference of yield was found between the seed treatments considering all 46 field tests (Total, field average, one-way ANOVA: $p<0.00002$). The overall hierarchy of treatments (effect on yield) was Ba2.5>Ea2.5>NoT. The difference observed between the two plant extracts (all 3 zones) was also significant: p(Ea2.5): $p<0.03$. However, in one zone (C: South-Eastern) comparison of the two extracts turned out to contrast the overall picture: the strongest effect in zone C was observed for *E. alba* extract (+27.7%) compared to *B. aegyptiaca* extract (+22.8%). Note that the same hierarchy of treatments was found when tested on formally produced seeds in the same zone (Table 2). Particularly, the results observed in Zones A and C appeared to be contrasting with regard to the hierarchy of the two plant extracts. Both zones were low yielding (Table 3). Also emergence (No Treatment) was low in both Zones A and C, despite the rainfall being substantially higher in Zone C (Table 4). Note that both plant extracts showed a similar overall effect on emergence (ca +6%). Strongest increases were observed in Zones C and A.

In conclusion, significant effects on yield were obtained for both extracts, but only in the two zones showing low baseline emergence (<65%) and low base line yield (<570 kg/ha). With regard to the effect on yield the overall hierarchy Ba2.5>Ea2.5 was found. However, particularly in zone C, the opposite hierarchy was observed (Ea2.5>Ba2.5) as previously observed using formally propagated seeds.

Antifungal effect on seed mycoflora

The direct antifungal effect of the two plant extracts, Ea2.5% and Ba2.5%, was compared on sorghum seeds carrying a moderate to high natural inoculum (6-40%) of three fungal genera: *Epicoccum*, *Curvularia* and *Fusarium* (Figure 2).

Two repetitions of blotter test show percentage of seeds infected by genera (A) *Epicoccum*, (B) *Curvularia* and (C) *Fusarium*. Each replicate consists of 100 formally produced seeds (Kapelga).

Figure 2 shows that both plant extracts cause inhibition on the genera *Epicoccum* and *Curvularia*, whereas only *B. aegyptiaca* extract inhibits *Fusarium*.

Protection of seedlings from fungal inoculation on seeds *in vitro*

Both plant extracts were compared with respect to their ability to protect seedlings of sorghum from a seed born fungal pathogen, *C. lunata* commonly found on sorghum seeds in Burkina Faso (Zida et al., 2008a; Stokholm et al., 2016). Seeds of variety Kapelga (sample 49.071) were inoculated with either water (control) or the fungus before being treated (NoT, H_2O, Ea2.5%, Ba2.5% and Hot Water (HW)). After treatment seeds were sown on hoagland medium, and shoots of seedlings were analyzed after 16 days (Figure 3).

From Figure 3, it is seen that the pathogenicity of *C. lunata* (inhibition of growth) can to some extent be counteracted by treatment with either of the two plant extracts. The extract from *E. alba* appears as the most protective ($p<0.05$). Both plant extracts are also protective compared to pure water ($p<0.05$). In contrast, no significant differences in shoot weight are found for treatment of non-inoculated seeds with plant extracts.

Protection of seedlings grown from naturally infected, farm-saved seeds

Farm-saved seeds from the village, Diapangou (South-Eastern zone) were tested in seed treatment *in vitro*. Both growth (shoot weight) and seedling health (roots scored for absence of dark necrosis) were recorded (Figure 4).

From Figure 4 it can be seen that with respect to seedling growth, the same hierarchy was observed for farm-saved seeds (naturally infected) as for artificially inoculated seeds above (Figure 3). However, for the two plant extracts only treatment with *E. alba* extract resulted in a significant difference in growth compared to hydropriming (p<0.05). On the other hand, both extracts suppressed development of root necrosis (symptom of fungal infection) significantly compared to hydropriming (p< 0.05).

DISCUSSION

In this study, we have found significant, field averaged yield increases caused by plant extracts of *E. alba* and *B. aegyptiaca*, applied on both farm-saved and formally produced seeds. A yield enhancing effect of both plant extracts was found in comparison to hydropriming. Regional differences in the crop response to seed treatments were observed and significant effects were observed only in two zones (out of three) where the lowest baseline yield and the lowest crop emergence were also found. Tests *in vitro* confirmed the antifungal effect of the two plant extracts both showing inhibition of seed mycoflora and the ability to protect seedlings from seed-borne inoculum of pathogenic fungi.

Water alone: Hydropriming

Hydropriming of seeds is a well-known method to improve yield of crops sown by hand in semi-arid, tropical agriculture (Harris, 2006; Navaz et al., 2013). Yield increases of typically 20 to 30% are observed in several crops, when hydropriming is applied in semi-arid Western Africa (Aune et al., 2017). Specifically in sorghum, the method has been documented several times using soaking times of 8 to 10 h in field trials (Ramamurthy et al., 2005; Aune and Ousman, 2011; Aune et al., 2012; Abdalla et al., 2015). Using 6 h of soaking time, it was recently found that a 19.6% mean increase of sorghum yield could be obtained by hydropriming across three locations (villages) selected for testing in Burkina Faso. The level of yield enhancement by hydropriming found for sorghum in this study appeared to be at the same level (+15.1%, Table 1). No zonal difference in the effect of hydropriming was observed between the two zones B and C compared in Table 2.

Plant extracts and water

Several plant extracts have previously been shown to improve seedling growth and even the yield of sorghum by seed treatment (Raghavendra et al., 2007; Tegegne et al., 2008; Manjunatha et al., 2013; Andresen et al., 2015; Ahmad et al., 2016). In most cases, antifungal activity has been indicated as a possible mechanism of action (Koch and Roberts, 2014). The finding in the present study of an aqueous extract from the bark of *B. aegytiaca* providing a significant yield increase (Ba2.5>H_2O>NoT, Table 1), is to our knowledge, the first demonstration of a specific yield enhancing effect of this plant in a field trial. Although this result was only significant for field averaged values, we consider the conclusion as robust, noting that the absolute levels of yield for non-treated seed varied considerably between individual fields (from 130 to 2800 kg/ha) making statistical treatment of absolute values difficult and strongly prone to a bias from the most high yielding fields. When the subset of data including treatment with *E. alba* extract was stratified into two zones (Table 2) it became apparent that only in the low yielding Zone C, a specific, yield enhancing effect of both plant extracts was observed (Ea>Ba>H_2O>NoT). In this zone, the *E. alba* extract was observed as more efficient (+29%) in comparison to the *B. aegyptiaca* extract (+20%) and hydropriming (+11%). The difference in comparison to hydropriming was significant. For *E. alba,* an additional effect compared to water has been found before in sorghum (Zida et al., 2012, 2015, 2018). However, the present study is the first to test a moderate/low concentration of *E. alba* extract (2.5% w/v) against both non-treated and hydroprimed seeds in parallel under field conditions. In conclusion, when testing formally propagated seeds, a specific effect on yield relative to pure water was found for both plant extracts. The strongest effects were observed in Zone C, which is in agreement with previous findings comparing Kindi (Zone B) and Diapangou locations (Zone C), (Zida et al., 2016).

Farm-saved seeds in three zones

Overall, the participatory trial showed that the initial findings of a significant effect on yield for both plant extracts on formally produced seeds could also beextended to farm-saved seeds (Table 3). Interestingly, the extract from *B. aegyptiaca* was significantly more efficient than the *E. alba* extract (31% compared to 21% yield increase across all zones). The South-Eastern zone (C) appeared as an exception since *E. alba* extract was observed as the most efficient in this zone (Ea: +28%, Ba:+23%) as also observed for formally propagated seeds (Ea: 29%; Ba: 20%, Table 2). Contrarily, in the Northern Zone (A), the observed difference between the two extracts was substantial and in favor of *B. aegyptiaca* (Ea: +18%, Ba: +42%, Table 3). The latter difference was statistically significant. Altogether, our field data were strongly indicative of the existence of regional differences with regard to the seed treatment response and indicated functional biochemical or biological differences between the two plant extracts. Existence of regional/geographical differences for treatment with *E. alba* extract is in good agreement with our previous findings including a low level of emergence found in the zone showing the strongest response (Zida et al. 2016). Interestingly, Zone C showed a low level of emergence and yield despite a relatively high level of rain fall, indicating that biotic factors could play a role (Tables 3 and 4). In the trial with farm-saved seeds, the effect on emergence was almost the same for the two plant extracts, whereas the effect on yield was significantly different. This might indicate that species-specific, biochemical activities of the plant extracts (such as antifungal activity), are having a substantial effect particularly on post-emergence growth of the treated crops.

Results *in vitro*

Antifungal activity has been attributed to both *E. alba* (Abdel-Kader et al., 1998; Saraswathy and Kumaran, 2012; Banaras et al., 2015) and B*. aegyptiaca* (Chapagain et al., 2007; Bonzi et al., 2012). In the present study, results *in vitro* confirmed the antifungal effect of both extracts in two ways.

Mycoflora on seeds:

A reduction of fungi from the genera, *Epicoccum* and *Curvularia*, was repeatedly observed for both extracts on treated seeds (blotter test, >2 fold reduction), whereas no reduction was observed for soaking in pure water (Figure 2). With respect to the genus *Fusarium*, a fundamental difference between the two extracts appeared to exist, since efficient elimination of this genus was observed for *B. aegyptiaca* extract but not for *E. alba* extract (Figure 2).

Effect on seedling growth and health:

Compared to hydropriming, a significant effect of the two plant extracts on growth was only observed for seedlings grown from seeds inoculated with a fungal pathogen. Protection from the pathogen inoculum, *C. lunata*, was most efficiently exerted by the *E. alba* extract (Figure 3). For farm-saved seeds carrying a natural inoculum of fungi (*Epicoccum*, *Curvularia*, *Fusarium*) the frequency of healthy roots (without visible necroses) increased from 30% for soaking in pure water to 70% or more for both plant extracts (p<0.05).

In conclusion, both mycoflora on seeds and symptoms caused by fungal inoculum on seeds could be reduced by treatment with the plant extracts. Some differences appeared to exist between the two plant extracts with regard to inhibition of *Fusarium* and protection from *C. lunata*.

Perspectives

The finding that treatment of farm-saved seeds with either of two antifungal plant extracts leads to overall, significant yield increases is encouraging for proceeding towards implementation of the technology in Burkina Faso. It is further encouraging that both plant species are commonly found in the regions where their activity appears to be optimal, respectively (*E. alba* in South-East; *B. aegyptiaca* in the North). *E. alba* is already well known to farmers due to its medicinal characteristics and no acute toxicity of an extract from *E. alba* leaves was found in a recent study (Udayashankar et al., 2016). With regard to the *E. alba* extract implementation in the South-Eastern zone therefore appears to be straight forward. With regard to *B. aegyptiaca* the somewhat surprising finding of a 42% yield increase on farmers' fields in the Northern region strongly encourages a focus on implementing the use of this extract in the Northern region where the desert tree, *B. aegyptiaca*, is also commonly found. However, careful consideration should be made regarding a safe protocol for obtaining the bark powder without killing the tree leading to unintended deforestation. More research into the differences between low-yielding/dry versus low-yielding/humid zones on the importance of hydropriming and yield loss caused by different ascomycetes seems justified by the present observations of a difference in crop response to treatments. Local seeds should be given a high priority in such research. In addition, experiments to domesticate *E. alba* and *B. aegyptiaca* by farmers could be initiated to increase the feasibility of sampling botanical material.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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